

Applicants: Vadiraja Murthy and Edward R. Burns
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Applicants request that the following amendments be made in the above-identified application:

In the Specification:

Page 1, after the title and before the Background of the Invention, please ~~delete~~ the sentence beginning "This application is" and insert the following sentence: --This application is a continuation of U.S. Application No. 08/421,079, filed April 13, 1995, now abandoned.--

In the Claims:

Please cancel Claims 1-4, 8-10 and 19.

Please add the following new Claims 20-23.

--20. (new) A method for diagnosing erythrocyte hemolysis in a subject comprising the steps of:

(a) obtaining a serum sample from said subject; and
(b) detecting the presence of erythrocyte adenylate kinase in said sample, the presence of said erythrocyte adenylate kinase being indicative of erythrocyte hemolysis in said subject.--

--21. (new) A method for detecting the presence of hemolyzed erythrocytes in a serum sample comprising the steps of:

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(a) electrophoresing said serum sample in a gel matrix so that erythrocyte adenylate kinase migrates to a known location on said gel matrix;

(b) contacting said gel matrix with an adenylate kinase-specific visualization reagent which reacts with said erythrocyte adenylate kinase and causes emission of fluorescence upon exposure of said gel matrix to ultraviolet light;

(c) exposing said gel matrix to ultraviolet light; and

(d) detecting emission of fluorescence at said known location on said gel matrix, emission of said fluorescence at said known location being indicative of hemolyzed erythrocytes present in said serum sample.--

--22. (new) The method of Claim 21, wherein said adenylate kinase visualization reagent comprises adenosine diphosphate, D-glucose, nicotinamide adenine dinucleotide, hexokinase and glucose-6-phosphate dehydrogenase.--

--23. (new) A method for determining erythrocyte adenylate kinase enzymatic activity in a serum sample comprising the steps of:

(a) determining total adenylate kinase enzymatic activity in a first aliquot of the serum sample by mixing the first aliquot with a first adenylate kinase-specific visualization reagent which reacts with the total adenylate kinase causing a

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change in absorbance of the mixture, and measuring the change in absorbance of the mixture, the change in the absorbance being indicative of the total adenylate kinase enzymatic activity;

(b) calculating the percent of the erythrocyte adenylate kinase in the total adenylate kinase in the serum sample by:

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- (1) electrophoresing a second aliquot of the serum sample in a gel matrix so that the erythrocyte adenylate kinase migrates to a known location on the gel matrix;
 - (2) contacting the gel matrix with a second adenylate kinase-specific visualization reagent which reacts with the total adenylate kinase and causes emission of fluorescence upon exposure of the gel matrix to ultraviolet light;
 - (3) exposing the gel matrix to the ultra-violet light;
 - (4) measuring total fluorescent light emitted from the gel matrix;
 - (5) measuring fluorescent light emitted from the gel matrix at the known location of the erythrocyte adenylate kinase migration on the gel matrix; and
 - (6) calculating the percent of the erythrocyte adenylate kinase by dividing the measured fluorescent light of step (b) (5) by the measured total fluorescent light of step (b) (4); and
 - (c) multiplying the percent of erythrocyte adenylate kinase by the total adenylate kinase activity to give the erythrocytic adenylate kinase enzymatic activity in the serum sample.--